Exhibit F

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Generation of a bovine oocyte cDNA library and microarray: resources for identification of genes important for follicular development and early embryogenesis.

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Abstract

The oocyte is a key regulator of ovarian folliculogenesis and early embryonic development. However, the composition of the oocyte transcriptome and identities and functions of key oocyte-specific genes involved in the above processes are relatively unknown. Using a PCR-based cDNA amplification method (SMART technology), we constructed a bovine oocyte cDNA library. Analysis of 230 expressed sequence tags (ESTs) from this library identified 102 unique sequences. Although some correspond to housekeeping genes (e.g., ribosomal protein L15) and some represent genes previously known to be expressed in oocytes and other tissues, most encode for genes whose expression in mammalian oocytes has not been reported previously (e.g., cocaine- and amphetamine-regulated transcript) or genes of unknown function. Sixteen did not show significant sequence similarity to any entries in the GenBank database and were classified as novel. Using over 2,000 unsequenced, randomly selected cDNA clones from the library, we constructed an oocyte microarray and performed experiments to identify genes preferentially expressed in fetal ovary (an enriched source of oocytes) relative to somatic tissues. Eleven clones were identified by microarray analysis with consistently higher expression in fetal ovaries (collected from animals at days 210-260 of gestation) compared with spleen and liver. DNA sequence analysis of these clones revealed that two correspond to JY-1, a novel bovine oocyte-specific gene. The remaining nine clones represent five identified genes and one additional completely novel gene. Increased abundance of mRNA in fetal ovary for five of the six genes identified was confirmed by real-time PCR. Results demonstrate the potential utility of these unique resources for identification of oocyte-expressed genes potentially important for reproductive function.

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